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Research Note

Measurement of Metallic Ions in *Biomphalaria glabrata* (Gastropoda) Infected with *Echinostoma caproni* (Trematoda) and in Uninfected Snails

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ABSTRACT: Inductively coupled plasma atomic emission spectrometry (ICP-AES) was used to study metallic ions in whole bodies of uninfected *Biomphalaria glabrata* snails and those experimentally infected with larval *Echinostoma caproni* trematodes. Infected snails were analyzed at 6 wk postinfection when the digestive gland–gonad complex contained 100–200 daughter rediae per snail. Cohort snails that were left uninfected were analyzed at the same time as controls. Nine metals were detected in *B. glabrata* by ICP analysis as follows: boron, copper, iron, manganese, zinc, calcium, magnesium, sodium, and potassium. There were no significant differences (Student's *t*-test, $P > 0.05$) in the concentrations of these metals in whole infected versus whole uninfected snails.

KEY WORDS: Metallic ions, spectrometry, *Biomphalaria glabrata, Echinostoma caproni,* Trematoda, Gastropoda.

There is little information on the metallic ion content of *Biomphalaria glabrata* snails and no information on the effects of larval trematode infection on the metallic ion content of this snail. Gabrashanska et al. (1991) examined the effects of larval *Echinostoma revolutum* on the mineral composition of the freshwater snail *Lymnaea stagnalis.* They used whole snail bodies in their analysis and found significant differences in certain metallic ions between infected and uninfected snails. Another study, by Layman et al. (1996), examined the digestive gland–gonad complex (DGG) of *Helisoma trivolvis* snails naturally infected with larval *E. trivolvis* trematodes to determine the effects of parasitism on the metallic ion content of the snail. They found significant differences in certain metallic ions between infected and uninfected snails. Our laboratory has now examined the effects of larval parasitism by *E. caproni* on the metallic ion content of experimentally infected *B. glabrata* snails using inductively coupled plasma atomic emission spectrometry (ICP-AES).

B. glabrata snails were maintained at 22–24°C in aerated aquaria containing artificial spring water and exposed to *E. caproni* miracidia as described in Beers et al. (1995). Infected snails along with uninfected controls were maintained in aquaria as described in Beers et al. (1995) and used 6 wk postinfection. Snails were isolated individually to determine larval infection with *E. caproni* and then subsequently crushed to confirm the infection. Samples of infected snail bodies were pooled to achieve a wet weight of about 1 g (approximately 10 snails). Likewise, samples of uninfected snail bodies (about 10 snails) were pooled to obtain a similar wet weight. Five pools of infected snails and 6 pools of uninfected snails were prepared for each analysis. Prior to use in an analysis, each pool was rinsed several times with ultrapure (Milli-Q, Millipore, Bedford, Massachusetts) water and digested in boiling nitric acid. Each digested sample was diluted to 25.0 ml with 2% (v/v) nitric acid.

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Table 1. Mean ± standard deviation in mg/g of dry tissue of snails infected with *Echinostoma caproni* and uninfected snails as determined by ICP-AES (uninfected, $n = 6$; infected, $n = 5$).*

Element	Infected	Uninfected	Value of t
B	0.04 ± 0.04	0.04 ± 0.04	0.003
Ca	55 ± 11	59 ± 9	0.393
Cu	0.03 ± 0.02	0.03 ± 0.01	0.084
Fe	0.17 ± 0.05	0.18 ± 0.06	0.225
Mg	3.1 ± 0.4	3.2 ± 0.5	0.135
Mn	0.04 ± 0.01	0.04 ± 0.01	0.575
K	4.6 ± 0.5	4.1 ± 0.5	1.01
Na	1.2 ± 0.1	1.2 ± 0.3	0.509
Zn	0.09 ± 0.02	0.12 ± 0.06	1.26

* 95% confidence level: $t = 2.98$.

Sample solutions were analyzed for 26 elements by ICP-AES using a Thermo Jarrell Ash simultaneous-reading spectrometer with autosampler. The instrument was calibrated following EPA Method 6010A, which uses a 2-point calibration, a blank, and multielement standards. Interelement correction factors were employed to minimize any interference between elements in the samples. Reagent blank samples were also analyzed. Each sample, standard, and blank was analyzed using 3 30-sec integrations. The results for each sample were averaged and the reagent blank subtracted to calculate the final analysis values presented. A number of quality control checks were made during the analyses to verify the calibration curve, blank, and interelement correction factors.

Table 1 presents the data obtained from the ICP-AES analysis of 5 pools of infected and 6 pools of uninfected snails, each pool containing approximately 10 snails with a combined wet weight of 1 g. The elements that were not detected at concentration levels above the detection limits of the instrument were Al, Sb, As, Ba, Be, Cd, Cr, Co, Pb, Mo, Ni, P, Se, Ag, Sr, Tl, and V. Nine elements (B, Ca, Cu, Fe, Mg, Mn, K, Na, and Zn) were detected at concentrations above their detection limits. For each of these 9 elements, the means were compared for significant differences between infected and uninfected snails with Student's t-test. No statistically significant differences were found between whole snails (minus shells) infected with *E. caproni* and those not infected (Table 1).

Layman et al. (1996), using flame and graphite furnace atomic absorption spectrometry (AAS) and ICP-AES, showed changes in certain metallic ions as a result of larval *E. trivolvis* infection in the DGG of *H. trivolvis.* They found significantly higher amounts of Na and significantly lower amounts of Mg and Mn in the DGG of infected snails. Gabrashanska et al. (1991), using neutron activation analysis of metallic ion in studies of *Lymnaea stagnalis* snails infected with the larval trematode *Echinostoma revolutum,* found significantly higher concentrations of Ca, Na, Rb, and Sb and significantly lower concentrations of Ce, Cr, Cs, Cu, Fe, and Zn in the digestive glands of infected snails. Differences in metallic ions as a result of larval parasitism in the aforementioned studies reflect alterations in ionic balance and an influx of certain ions and an outflux of other ions from the larval trematodes to the snail host.

In the present study, we failed to detect any qualitative or quantitative differences with respect to metallic ions between *B. glabrata* infected with *E. caproni* and uninfected controls. Results of our study probably reflect in part the fact that whole snail bodies were used in the analyses rather than DGGs. Parasitism of *B. glabrata* by *E. caproni* is mainly confined to the DGG, which comprises about 25% of the mass of the snail. The pathochemical effects of the infection may have been diluted out in these analyses by examining the whole snail body rather than just the DGG.

There are few reports of the quantitative analysis of metallic ions in *B. glabrata.* Nduka and Harrison (1980) determined the concentrations of Ca, Mg, Na, and K in varous planorbid snails, including *B. glabrata,* by AAS. The use of ICP-AES allows the simultaneous quantification of 26 elements rather than the limited sequential determination of individual ions by AAS. In addition, ICP-AES provides detection limits that are generally lower than flame AAS. Therefore, we are able to report for the first time the trace-metal profile of *B. glabrata.* The 17 preceding elements were found to be present at concentrations below the detection limit of the instrument, which ranged from 0.02 to 5 mg/g of dry tissue. The concentrations of Ca, Mg, Na, and K (Table 1) in the bodies of snails were similar to those found earlier by AAS (Nduka and Harrison, 1980), which provides important confirmation of these values by an independent analytical method. The concentrations of B, Cu, Fe, Mn, and Zn in Table 1 are the first data reported for these elements in *B. glabrata* snails, and values

for Ca, Mg, Na, and K in *B. glabrata* infected with a larval trematode have not been reported before.

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Research Note

Gastrointestinal Helminths from Juvenile Red Drum, *Sciaenops ocellatus,* and Atlantic Croaker, *Micropogonias undulatus* (Sciaenidae), in East Matagorda Bay, Texas

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ABSTRACT: Juvenile *Sciaenops ocellatus* ($N = 20$) and *Micropogonias undulatus* ($N = 8$) from East Matagorda Bay, Texas, were examined for gastrointestinal helminths. A total of 7 parasite species or groups were recovered, 5 of which were common to both *S. ocellatus* and *M. undulatus.* No parasites were found to be unique to *M. undulatus,* although significant differences in the mean intensity of *Lecithaster confusus* (Hemiuridae) and unidentified cestode larvae were found. Differences in diet, which correlate with differences in mean length, between *S. ocellatus* and *M. undulatus* are believed to be the basis for observed differences in the mean intensity of gastrointestinal helminths.

KEY WORDS: *Lecithaster confusus, Bucephaloides* spp., Sciaenidae, red drum, *Sciaenops ocellatus,* Atlantic croaker, *Micropogonias undulatus, Diplomonorchis leiostomi.*

Although the red drum, *Sciaenops ocellatus* (Linnaeus), and Atlantic croaker, *Micropogonias undulatus* (Linnaeus), are important sportfish along the Gulf of Mexico coast, few studies have been conducted on their gastrointestinal helminths, and much of the information available results from survey reports. Particularly lacking is quantified data concerning parasitic infections in juvenile red drum and Atlantic croaker.

Many reports of parasites from red drum and Atlantic croaker are a result of the inclusion of these species in general surveys of fishes from a particular area. Loftin (1960) published an annotated checklist of trematodes and cestodes from northwest Florida and included a report of "*Bucephalopsis* sp." from *Sciaenops ocellata* in Alligator Harbor, Florida. Riggin and Sparks (1962) later identified this parasite as a new species, *Bucephaloides megacirrus,* and provided a full description including its occurrence in red drum from Grand Isle, Louisiana. Nahhas and Short (1965) published a list of the digenetic trematodes of fishes from Apalachee Bay, Florida, and

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